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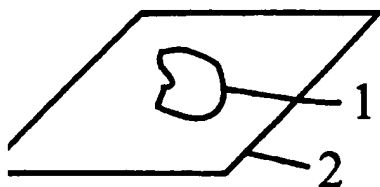
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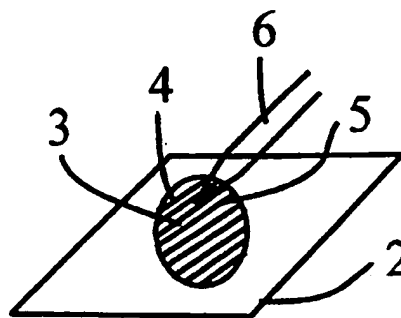
## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(54) Title: DROPLET REACTION CHAMBER



**Droplet without  
PETF- Powder**

**a**

**Droplet with  
PETF- Powder**

**b**

## (57) Abstract

A reaction chamber is constructed of a reactant-containing aqueous solution (1), which may be in droplet form, coated with a fluoropolymer powder such as Polytetrafluoroethylene (PTFE). Using micropipette, size of the droplet chamber (5) is increased or decreased by removing or adding liquid. A droplet chamber (5) containing a first reactant such as an enzyme-bound bead is combined with a second droplet chamber containing a second reactant to react the first and second reactants. A dialysis chamber contains a filtration membrane in contact with the droplet chamber (5). Placing the droplet chamber (5) between two electrodes and producing current flow between the electrodes provides an electrochemical reaction. A temperature-sensitive reaction such as Polymerase Chain Reaction (PCR) can be performed by heating or cooling the droplet chamber (5). In tissue culture, coating (4) a solution containing tissue cells with the fluoropolymer powder reduces sticking of the cells to the surfaces of a container.

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TITLE: DROPLET REACTION CHAMBER

FIELD AND BACKGROUND of THE INVENTION

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FIELD OF THE INVENTION

The present invention relates in general to chambers for chemical reactions, and in particular to the construction of such chambers by application of a fluoropolymer (polymer containing fluoro group), such as Polytetrafluoroethylene (PTFE) to aqueous solutions.

The invention described and claimed herein comprises a novel chemical reaction chamber for carrying out reactions in aqueous solutions, characterized by an outer coating of a fluoropolymer such as PTFE.

BACKGROUND INFORMATION

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Chemical (including biochemical) reactions are frequently carried out within a chamber or other container. In chemical and biochemical experiments, especially those involving small volumes (microvolumes) of a sample, there is always a significant loss of the sample as it disperses on the container walls.

Additionally, in reactions which are temperature sensitive, as for example the Polymerase Chain Reaction (PCR), there is also a loss of energy and time resulting from the need to heat (or cool) the reaction chamber itself.

30

However, when water or an aqueous solution contacts PTFE powder, it forms droplets coated with the powder. These droplets form a reaction chamber which does not involve significant loss of sample or significant energy or time loss  
5 from the heating or cooling of a reaction chamber.

If a solid insoluble material like active charcoal, metal powder, silica powder or a small piece of membrane or any other material is inserted inside or on the surface of the  
10 droplet reaction chamber, it will remain inside or on the surface.

By using a micro-pipette, liquid can be easily transferred from one droplet reaction chamber to another. The sizes of the  
15 droplets can be increased or decreased using a micropipette and eventually either by adding or removing the solution to/from the droplet reaction chamber.

These droplet reaction chambers can be used for different  
20 applications in chemical and biochemical reactions. In research laboratories, there are very often situation, when the container of the fluid can create problems while working with small volumes (in microliter range). The droplet reaction chambers can be prepared in larger sizes by simply shaking the  
25 aqueous solution with the PTFE powder.

#### OBJECTS

30 The foregoing problem is overcome, and other advantages are provided by a chemical reaction comprising an aqueous solution coated with a fluoropolymer, such as PTFE powder.

Among the objects of the present invention are to provide a new and useful chemical reaction chamber which does not rely on conventional walls to contain the chemical reaction and  
5 thereby to provide a new and useful chemical reaction which reduces loss of reactants and reduces the energy and time required to heat or cool the reaction.

These and other objects which will be apparent from the  
10 discussion which follows are achieved, in accordance with the invention, by providing a novel chemical reaction chamber for carrying out reactions in aqueous solutions, characterized by an outer coating of a fluoropolymer, such as PTFE.

15 The various features of novelty which characterize the invention are pointed out with particularity in the claims annexed to and forming a part of this disclosure. For a better understanding of the invention, its advantages and objects, reference is made to the accompanying drawings and descriptive  
20 matter in which a preferred embodiment of the invention is illustrated.

#### BRIEF DESCRIPTION OF THE DRAWING

25 The foregoing and still other objects of this invention will become apparent, along with various advantages and features of novelty residing in the present embodiments, from study of the following drawings, in which:

30 Figures 1a and 1b are a plan view of a droplet reaction chamber, contrasting an untreated droplet (Figure 1a) with a treated to form a droplet reaction chamber (Figure 1b).

Figure 2 illustrates a droplet reaction chamber with a membrane.

- 5 Figure 3-illustrates a droplet reaction chamber with solid particles.

Figures 4a and 4b illustrate the fusion of two droplet reaction chambers (Figure 4a) into a single droplet reaction chamber (Figure 4b).

#### DESCRIPTION OF THE PREFERRED EMBODIMENT

Referring to the drawings, the invention is a novel chemical reaction chamber for carrying out reactions in aqueous solutions, characterized by an outer coating of a fluoropolymer, shown in overview in Figure 1. The operation of the invention is described with reference to a commonly available, inexpensive fluoropolymer, the perfluorocarbon PTFE, available from DuPont under the tradename TEFLON™. It has been found that a preferred particle size is less than 500 microns. This is by way of illustration only, and the invention could be implemented using any suitable fluoropolymer.

25 As shown in Figure 1a, an aqueous solution (1) normally spreads on the support surface (2). However, when an aqueous solution containing reactants of interest (3) is coated with a coating layer (4) of PTFE, such as DuPont TEFLON™, it forms a droplet reaction chamber (5).

30

The coating (4) causes the aqueous solution (3) to remain stably in a defined volume without the need for any external solid wall (except for a support {2}).

- 5 The explanation of the operation of the invention is believed to be as follows. Due to their high affinity to form hydrogen binding, the terminal fluorine atoms in a fluoropolymer, such as PTFE, form hydrogen bonds with H-atom of water thus increasing the surface tension of the aqueous phase and
- 10 enabling the formation of spherical droplets. This explanation is reinforced by the observation that the addition of alcohol or detergents to the aqueous phase destroys the droplet reaction chamber. It is believed that the addition of alcohol decreases the hydrogen-binding of H-atoms of water with
- 15 fluorine atoms of PTFE, thus lowering the surface tension of the aqueous phase and resulting in disappearance of the droplet reaction chamber. Furthermore, it has been observed that the formation of droplet reaction chambers does not take place on the surfaces that contain OH-, or similar groups on
- 20 the surface and thus would disturb the hydrogen-bonding of PTFE and water., for example, glass. Similarly, detergents lower the surface tension of aqueous phase and have a tendency to accumulate preferentially at the surface thus reducing the formation of hydrogen-bonds between fluorine atoms of PTFE
- 25 And hydrogen atoms of water. Therefore, addition of any substance which reduces the surface tension will reduce or completely hinder the formation of droplets.

- On the other hand, molecules which increase the surface
- 30 tension of aqueous solution can enhance the formation of hydrogen binding between fluorine atoms of PTFE and OH-group of water. For example, solutes such as ionic salts increases



the surface tension of aqueous solutions as compared to the surface tension of pure water. However, this increase of surface tension by addition of ionic salts is significantly smaller as compared to the decrease in surface tension by  
5 addition of detergents or alcohol. Therefore, materials which do not contain OH- or other groups that negatively influence the surface tension of aqueous solutions, will be suitable as supports for droplet reaction chambers.

10 PTFE is an ideal substance for the described uses, since it is non-toxic, chemically inert, and able to withstand extremes of heat and cold.

The reactants may be combined, then coated, or one reactant  
15 may be coated, and additional reactants introduced through the PTFE layer (4). One method of doing so is by insertion using a pipette (6).

Similarly, a sample of the reaction product may be withdrawn  
20 through the PTFE layer (4), using for example a pipette.

Referring to Figure 3, in addition to reactants, the droplet reaction chamber (5) may contain solid particles, such as activated charcoal, a solid matrix for affinity purification,  
25 enzyme bound beads for biochemical reactions, cells or tissue, either within (7) the droplet reaction chamber (5) or on its surface (8). Further processing of the droplet reaction chamber is possible. Examples including electrophoresis, electroelution and electrochemical reactions, which may be  
30 accomplished by simply placing the droplet reaction chamber between electrodes. Addition of metallic powder to the droplet

reaction chamber increases the current flow through the chamber.

The results of the chemical reaction may likewise be further  
5 processed, for example as follows. The droplet reaction chamber may be destroyed either by the addition of a detergent, an organic solvent or any other substance that reduces surface tension. The PTFE coating may then be removed from aqueous component by centrifugation, leaving the reaction  
10 product.

Alternatively, the droplet reaction chamber may be dried and subsequently reformed by the addition of water.

15 Specific applications of the invention will now be illustrated.

Example 1. Use as a chemical or biochemical reaction chamber.

20 By pipetting an aqueous solution, buffer or reaction mixture on PTFE powder, a droplet reaction chamber is formed. The droplet reaction chamber can be broken in different aliquots without the loss of materials. The reactions inside the droplet reaction chamber can be started or blocked by  
25 pipetting appropriate chemicals, such as biochemicals, enzymes or inhibitors, into the droplet reaction chamber. Once the reaction is completed, the droplet reaction chamber can be centrifuged in a container containing a filter having pores smaller than the particle size of the PTFE.

30

Example 2. Application in Equilibrium Dialysis

Referring to Figure 2, if a membrane (9) is placed on a droplet reaction chamber (5), it floats along with the droplet reaction chamber (5). The lower surface of the membrane will be in contact with the liquid inside the droplet reaction chamber. A Liquid (10) may then be placed on the upper side of the membrane for the dialysis. Once equilibrium has been reached, a sample may be taken from either side of the membrane using for example a micropipette. Thus, the droplet reaction chamber may be used as a disposable dialyser for small sample.

#### Example 3. Combining Multiple Reaction Chambers

Referring to Figure 4, two or more reactants may be combined as follows. Each reactant is coated with PTFE as described above. The resulting droplet reaction chambers may then be combined by forcing them together, resulting in rapid mixing of their contents. In Figure 4a, two reactants are coated to form droplet reaction chamber 11 and 12, then combined to form droplet reaction chamber 13, in which a reaction between the contents of droplet reaction chamber 11 and 12 will occur, as shown in Figure 4b.

#### Example 4. Rapid Temperature Change.

In some reactions, notably PCR, it is necessary to rapidly vary the temperature of the reactants. Using a droplet reaction chamber, the heat and time otherwise expended on changing the temperature of a solid container is necessary.

#### Example 5. Application in Tissue Culture Experiments

Many natural cells and cell lines have a tendency to stick to the surface of containers. By using gently shaking a mixture of an aqueous solution containing such cells with PTFE powder, the tendency to stick is reduced. Cells may be grown inside  
5 the PTFE-coated aqueous phase suspension. In some cases, cell growth may be improved because of the three dimensional nature of the aqueous phase. A gentle shaking of the tissue culture container can evenly distribute the cells inside the chamber.

10 As can be seen from the above description and examples, it is possible to carry out a wide variety of chemical and biochemical reactions using the invention, simply providing appropriately sized droplets of an aqueous solution containing the desired reactants and by coating the droplets with PTFE.

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Thus, there has been described a novel chemical reaction chamber for carrying out reactions in aqueous solutions, characterized by an outer coating of PTFE, that has a number of novel features, and a manner of making and using the  
20 invention.

While a specific embodiment of the invention has been shown and described in detail to illustrate the application of the principles of the invention, it will be understood that the  
25 invention may be embodied otherwise without departing from such principles and various modifications, alternate constructions, and equivalents will occur to those skilled in the art given the benefit of this disclosure. Thus, the invention is not limited to the specific embodiment described  
30 herein, but is defined by the appended claims.

## CLAIMS:

I claim:

1. A chamber for carrying out chemical reactions in aqueous  
5 solution, comprising a droplet reaction chamber, said  
droplet reaction chamber comprising: an aqueous solution  
containing at least one reactant in droplet form; and a  
coating of a fluoropolymer powder on the said droplet,  
which maintains said aqueous solution in droplet form.
- 10 2. A chamber as in claim 1, further comprising a support for  
supporting said droplet reaction chamber.
3. A chamber as in claim 1 wherein said fluoropolymer powder  
is Polytetrafluoroethylene (PTFE).
4. A chamber as in claim 1 wherein said fluoropolymer has a  
15 particle size of less than 500 micrometer.
5. A dialysis chamber comprising a droplet reaction chamber as  
in claim 1 and further comprising a filtration membrane in  
contact with said droplet reaction chamber
6. A method for carrying out chemical reaction, comprising  
20 the steps of:  
providing a first chamber as in claim 1 wherein said  
chamber contains a first reactant;  
providing a second chamber as in claim 1 wherein said  
chamber contains a second reactant; and  
25 combining the chambers.
7. A method as in claim 6 wherein said first reactant  
comprises an enzyme-bound bead.
8. A kit for carrying out chemical reactions comprising a  
fluoropolymer powder, a container and reagents.
- 30 9. A method for tissue culture comprising the steps of:  
providing an aqueous solution containing biological cells  
in a container and coating said solution with a

fluoropolymer powder whereby sticking of the cells is reduced to the container surface in contact with said solution.

- 10.A method for carrying out a temperature-sensitive chemical  
5 reaction, comprising the step of:  
providing a chamber containing a reactant as in claim 1 and  
heating or cooling said chamber to carry out said chemical  
reaction.
- 11.A method as in claim 10 wherein said temperature-sensitive  
10 chemical reaction is Polymerase Chain Reaction (PCR).
- 12.A method for chemical processing the steps of:  
providing a chamber containing a reactant as in claim 1;  
reacting said reactant to carry a chemical process within  
said chamber; destroying said chamber by adding a chamber-  
15 destroying substance; and removing the fluoropolymer powder  
by filtration and centrifugation.
- 13.A method as in claim 12 wherein said chamber-  
destroying substance is a detergent.
- 14.A method as in claim 12 wherein said chamber-destroying  
20 substance is an organic solvent.
- 15.A method for carrying out an electrochemical process  
comprising the step of:  
providing a droplet reaction chamber as in claim 1; placing  
said droplet chamber between electrodes; and providing a  
25 current flow between the two electrodes to produce an  
electrochemical reaction.
16. A method as in claim 15 wherein said electrochemical  
process comprises electrophoration.
17. A method as in claim 15 wherein said electrochemical  
30 process comprises electroelution.

18. A method as in claim 15 further comprising the step of placing metal particles within or on the surface of said droplet reaction chamber.

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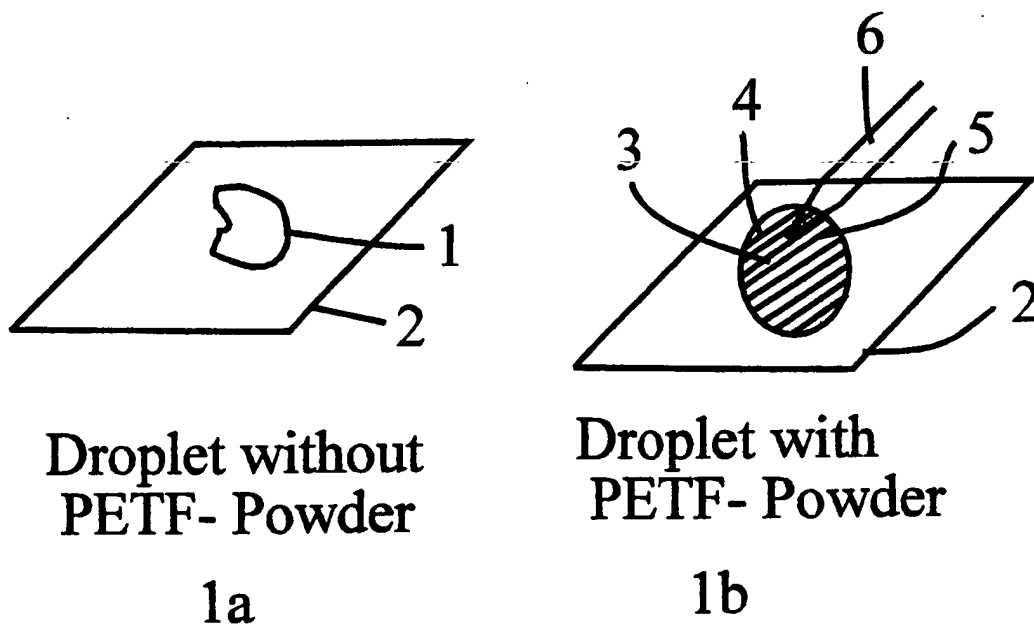


Fig. 1



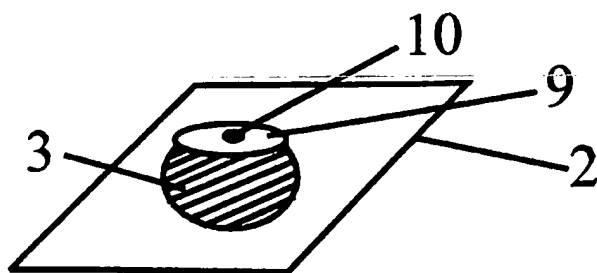


Fig. 2

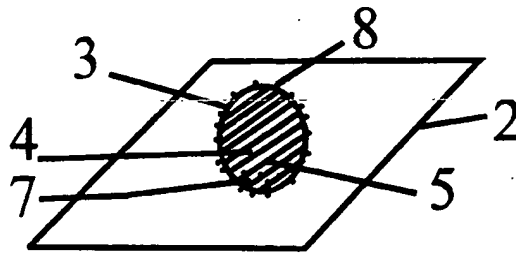


Fig. 3

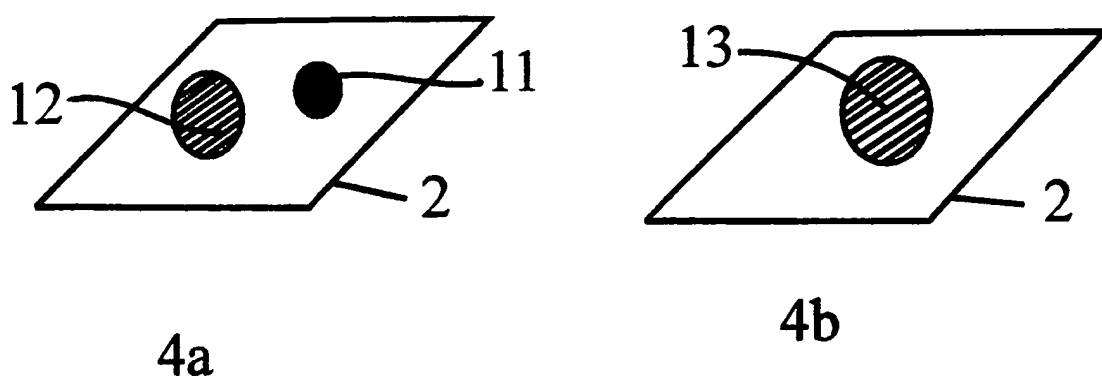


Fig. 4

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US98/12808

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C12P 1/00

US CL : 435/41

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/41, 174, 187, 180, 182, 173.1

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Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,401,634 A (MILBRATH) 28 MARCH 1995 (28.03.95) SEE ENTIRE DOCUMENT.	1-18
A	US 5,374,397 A (TERMIN) 20 DECEMBER 1994 (20.12.94) SEE ENTIRE DOCUMENT.	1-18
A	US 5,466,570 A (JAGANNATHAN ET AL) 14 NOVEMBER 1995 (14.11.95) SEE ENTIRE DOCUMENT.	1-18

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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